United States Environmental Protection Agency

LABORATORY ANIMAL PROJECT REVIEW

Please note:

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

LAPR Information

LAPR Title:	In utero effects of dipentyl phthalate, a potent endocrine disrupting chemical, on the reproductive development in the CD-1 mouse
LAPR Number:	18-04-003
Principal Investigator	Exemption 6
Author of this Document:	Exemption 6/RTP/USEPA/US
Date Originated:	04/07/2015
LAPR Expiration Date:	04/30/2018
Agenda Date:	04/22/2015
Date Approved:	04/29/2015
Date Closed:	04/17/2017

APPROVALS

APPROVER	NAME	APPROVAL DATE	COMMENTS	
	Exemption 6/RTP/USEPA/US by Exemption 6/RTP/USEPA/US	04/29/2015	Designated Member Reviewer	
	Exemption 6/RTP/USEPA/US by Exemption 6/RTP/USEPA/US	04/29/2015	DMR	
	Exemption 6 Exemption 6 Exemption 6/RTP/USEPA/US by Exemption 6/RTP/USEPA/US	04/29/2015	DMR	

Administrative Information

1. Project Title (no abbreviations, include species):
In utero effects of dipentyl phthalate, a potent endocrine disrupting chemical, on the reproductive development in the CD-1 mouse

Is this a continuing study with a previously approved LAPR?
Yes
Please provide the previous LAPR# 15-04-004

2. Programatic Information
a. What Program does this LAPR support? Please provide the Research Program, Project, Task Number and Title.
CSS 12.01 Adverse outcome pathway (AOP) discovery and development
b. What is the Quality Assurance Project Plan (QAPP) covering this project?
IRP-NHEERL-RTP/RTD/EB/Exemption/2008-001-r0

3. EPA Principal Investigator/Responsible Employee:

Principal Investigator	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	Exemption 6	TAD	MD
	Lotus Notes Address	*Branch*	
	Exemption/RTP/USEPA/US	RTB	

4. Alternate Contact:

Alternate Contact	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	Exemption 6	TAD	MD
	Lotus Notes Address	*Branch*	
	Exemption 6/RTP/USEPA/US	RTB	

SECTION A - Description of Project

1. Explain the study objective(s) in non-technical language such that it is understandable by non-scientific

persons. Explain how the benefits from the knowledge gained from this research outweigh the costs to the animals used in this research. If this is a continuing study from a previous LAPR, briefly justify the continuation. Please spell out all acronyms and abbreviations with their initial use.

Phthalates are plasticizers that give plastic and vinyl their flexibility; these chemicals are used in a wide variety of commercial products, including cosmetics, personal care products, toys, shower curtains, wallpaper, food packaging, wood finishes, detergents, medical devices, insecticides, and vinyl flooring. Thus, humans, including pregnant women and children, are exposed to multiple phthalates. Even though there are no known uses for dipentyl phthalate (DPeP), the Centers for Disease Control reported that about 29% of the urine samples contained DPeP metabolites, indicating widespread exposure.

Phthalates, especially DPeP, have been shown to produce serious reproductive tract malformations in male rats when administered in utero during the critical period of sexual differentiation. Similar effects of the phthalates have been reproduced by some laboratories using mice, whereas several other laboratories have reported that the fetal mouse does not respond to phthalates like the rat. It has been claimed that mice and possibly humans are insensitive to these phthalate effects, which raises some uncertainty in extrapolating the effects of chemicals in rats to humans. Towards improving the risk assessment of phthalates in general, and DPeP in particular, a goal of this project is to determine if DPeP adversely affects reproductive development in the mouse after in utero exposure.

In the previous LAPR, we have monitored the reproductive development of mice that were exposed to DPeP in utero and are now completing the necropsies of the male mice (now about 5 months of age and fully mature). The female mice have already been necropsied under the previous (expiring) LAPR and it is our intent to transfer the male mice to this current LAPR in order to complete the necropsy of the male offspring. The objective of this new LAPR is to complete the necropsy of adult mice exposed to DPeP in utero being carried over from the expiring LAPR.

Thus far, the results of the ongoing DPeP study indicate that the mouse response differs from the rat in that the mouse showed dose-related perinatal mortality, and skull and eye malformations at the high dose. Unlike the rat, the male mouse offspring showed no reductions in neonatal anogenital distance, no increases in female-like nipple retention, and thus far (as the necropsies have not been completed) no reproductive tract malformations.

2. Scientific rationale for proposed animal use.

a. Why is the use of animals necessary?

The process of in utero sexual differentiation cannot be studied in vitro or in silico at this time. Whole animal studies are essential to determine the effects of endocrine disrupting compounds (EDCs), singly or in mixture, on male and female fetal rats. The whole animal is needed for EDC research for some of the fetal effects are not manifested until puberty so the whole animal must develop to that stage.

b. Justify the species requested:

Mice are the species of choice since the mechanisms of sexual differentiation are extremely well understood, the strain we will use is an excellent breeder and the question we are trying to determine whether or not the mouse responds to EDCs like the rat.

3. How was it determined that this study is not unnecessary duplication?

We evaluate all of the scientific literature on each chemical as well as examine the study files submitted by industry to the regulatory agencies, when possible (information may be available on the EPA, Food and Drug Administration or World Health Organization websites or regulations.gov). There are no well conducted studies of the effects of varied dosage levels on fetal mouse sexual differentiation for most phthalates.

SECTION B - In Vivo Procedures

1. Briefly describe the experimental design. Include descriptions of the age, weight and sex of the animals. Supplementary information may be attached at the end of the LAPR, but please include critical information within the body of the LAPR.

In the previous LAPR, we exposed pregnant mice to DPeP from gestation days (GD) 8 to 17 of gestation

and allowed the dams to deliver. Postnatal measurements to date have included offspring litter sizes, anogenital distance, nipple retention, and onset of puberty. Pups were weaned at 25 to 30 days of age and housed in unisex groups of 3 to 5 mice per cage.

Under this new LAPR the mice will be transferred from the previous LAPR and necropsied within the next 60 days and reproductive organ weights and morphology examined. Mice will be euthanized quickly by decapitation and exsanguination; blood will be collected for measurement of testosterone.

2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number requested for the duration of the LAPR.
We will be transferring up to 80 males from the previous LAPR.

3. State how many animals over the study period are expected to be used under the following three categories of pain/distress (USDA nomenclature as defined in the instructions): Please enter numbers only.

Categories	Adults	Offspring
C) Minimal, transient, or no pain/distress:	80	
D) Potential pain/distress relieved by appropriate measures:		
E) Unrelieved pain/distress:		

4. Does this LAPR include any of the following:
☐ Restraint (>15 Minutes) ☐ Survival surgery
☐ Food and/or water restriction (>6 Hours) ☐ Non-survival surgery

5. Category C procedures. Describe each procedure separately, include details on the following:
a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):
None. (Animals were exposed in utero under the previous LAPR.)
b. Survival Blood Collections (method, volume, frequency):
none
c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):
none
d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:
none
e. Breeding for experimental purposes (e.g. length of pairing, number of generations):
none
f. Describe how animals will be identified and monitored. Include description of identification procedures. (For example, if transponders are used, how are the animals prepared?) Include frequency of observations and by whom:
Litters are identified by cage numbers, males within litters are identified with markings. They are being monitored several times a week by the lab staff.

6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).
a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):

b. Blood Collection (Provide a description of the procedure including method, volume, and frequency if appropriate. Indicate if the procedure is survival or terminal. Include preparatory methods, descriptions of incisions, etc.):

c. Testing methods:

d. Restrictions placed on the animals' basic needs (e.g., food and/or water restriction, light cycles,

temperature). Provide details regarding the length of restriction. Describe the method(s) for assessing the health and well-being of the animals during restriction. (Amount of food or fluid earned during testing and amount freely given must be recorded and assessed to assure proper nutrition.):

e. Describe how animals will be monitored (e.g., frequency of observations, by whom):
f
f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:

g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:

7. Surgical Category D and E procedures. Indicate if the surgery is survival or terminal. Describe each surgical procedure separately, include details on the following (Also fill in Section B.9)

a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:

b. Anesthetic regimen (Drugs, dosages, volume, route of administration and delivery schedule). The use of paralytic or neuromuscular blocking agents w/o anesthesia is prohibited:

c. Postoperative care (thermal support, special feeding, responsible personnel, removal of sutures/staples, frequency and duration of monitoring including weekend and holiday care):

d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):

e. Will any animal be subject to more than one surgical procedure over the course of its lifetime, either here at NHEERL or elsewhere?
◯ Yes ◯ No
f. Identify any surgical procedures performed at other institutions or by vendors:

8. Humane interventions (for treatments/procedures in all categories).

a. What resultant effects, if any, do the investigators expect to see following procedures or treatment? Please include transitory as well as permanent effects. Examples might include lethargy, ataxia, salivation or tremors. Indicate the expected duration of these effects.
Some of the high dose exposed mice have needed teeth clipping due to malocclusions and gel food supplement has been provided. The skeletal effects were not anticipated based upon what we had seen at these dosage levels in male rats.

b. State the criteria for determining temporary or permanent removal of animals from the study. Describe actions to be taken in the event of deleterious effects from procedures or chemical exposures. Describe actions to be taken in the event of clinical health problems not caused by procedures or exposures.
Extreme weight loss, excessive salivation, excessive lacrimation, excessive defecation, extreme lethargy or torpor, tremors or any other sign of overt toxicity would result in removal of animals from the study.

9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.
NA

SECTION C - Animal requirements

Describe the following animal requirements :

1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.

a. Animals to be purchased from a Vendor for this study: 0

b. Animals to be transferred from another LAPR: 80

LAPR Number that is the source of this transfer:

c. Animals to be transferred from another source:

d. Offspring produced onsite (used for data collection and/or weaned):

e. TOTAL NUMBER of animals for duration of the LAPR 80

2. Species (limited to one per LAPR): Mouse/Mice

3. Strain: CD-1 mouse/mice

Describe special requirements for animals with altered physiological responses (e.g., genetically altered, aged)

Animals with malocclusion have had teeth clipped and fed gel food supplement

4. Sources of animals:

Previous LAPR 15-04-004. (Dams of these animals were from Charles River Laboratories.)

5. Provide room numbers where various procedures will be performed on animals:

6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be reviewed.

Exem
Exem
Exem

Room Numbers:

7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)

none

8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.

9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)

none

10. Housing and Enrichment.

The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.

The adult male offspring are being housed in groups of 3 to 5 mice per group in clear plastic cages with pine shavings as bedding. The mice should not be given any materials that might contain plastic, pesticides or metals, especially plastics since many plastics contain phthalates and other EDCs.

SECTION D - Agents Administered to Animals

1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used.
Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for dosing.

None. (Dosing with dipentyl phthalate was done to the dams under the previous LAPR.)

2. Describe compounds to be administered to animals.

a. Are all substances pharmaceutical grade? If not, provide a scientific justification for the use of non pharmaceutical grade compounds.

NA

b. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in the LAPR. Provide information to assure that such material is pathogen free. Indicate what safety precautions are necessary for handling the material.

NA

c. Provide a statement regarding any safety precautions necessary for handling any of these materials.

NA

NOTE: Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.

SECTION E - Personnel Training and Experience

1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are giving assurance that these personnel have received all required training and are qualified to perform the Category C techniques requested.

Use this area to type in additional personnel information not available in the table drop-down lists:

Hint: *The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).*

NAME	ROLE	SPECIFIC RESPONSIBILITY	RELEVANT TRAINING
Exemption 6	Principal Investigator	necropsy analyze data	all required EPA training and 25 years of animal use under approved protocols
Exemption 6	Technical Staff	design, dose,necropsy, analyze data	all required EPA training and 10 years of animal use under approved protocols
Exemption 6	Technical Staff	design, dose,necropsy, analyze data	all required EPA training and 4 years of animal use under approved protocols
Exemption 6	Technical Staff	dose,necropsy	all required EPA training and several months of animal use under approved protocols
Exemption 6	Post-Doc	design, dose,necropsy,	all required EPA training and several months

		analyze data	of animal use under approved protocols
RTP-NHEERL	Tech Support	Category C Procedures	All NHEERL required training is complete.

SECTION F - Animal Breeding Colonies

This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.

Describe:

1. Estimated number of breeding pairs and liveborn per year

2. Breeding protocols and recordkeeping

3. Methods for monitoring genetic stability

4. Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR

SECTION G - Euthanasia

1. When will the animals be euthanized relative to experimental procedures?

As fully mature offspring at about 6 months of age

2. Describe the euthanasia techniques:

Method(s): Decapitation plus exsanguination, A spare guillotine will be available

Agent(s):

Dose (mg/kg):

Volume:

Route:

Source(s) of information used to select the above agents/methods:

2013 AVMA Guidelines on Euthanasia.

3. Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing more than 200 grams).

4. Describe how death is to be confirmed.

Vital organ section, Prolonged absence of breathing

SECTION H - Disposition of Used and Unused Animals

Describe the disposition of any animals remaining after project completion.

Euthanized as above

The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?

○ Yes ● No

SECTION I - Assurances

1. Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.
2. All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.
3. Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.
4. The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.
5. The IACUC and Attending Veterinarian will be promptly notified of any unexpected study results that impact the animals' well-being, including morbidity, mortality and any occurrences of clinical symptoms which may cause pain or indicate distress.
6. All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.
7. I certify that I am familiar with and will comply with all pertinent institutional, state and federal rules and policies.
8. The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.

EPA Principal Investigator	Certification Signature Date
Exemption 6 Exemption 6	04/07/2015

Submitted: 04/07/2015

Certification:

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	***Approval Date***	***Phone Number***	***Division***	***Mail Drop***
Exemption 6	04/07/2015 by Exemption 6 Exemption 6 Exemption 6 Exemption 6 Exemption 6 /RTP/USEPA/US	Exemption 6 ***Lotus Notes Address*** Exemption 6 Exemption 6 Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US	TAD ***Branch*** RTB	MD ***Submitted to Branch Chief for Approval*** 04/07/2015 02:58 PM

ATTACHMENTS

Amendment for Effects of individual endocrine disrupting chemic mouse dpep postnatal added 12 11 2014.pdf

Actions

First Update notification sent: 03/09/2016
Second Update notification sent: 04/13/2016
First 2nd Annual notification sent:

Second 2nd Annual notification sent:

1st Expiration notification sent:
2nd Expiration notification sent:

History Log: